American Public Health Association

SOME OBSERVATIONS ON METHODS FOR THE DETECTION OF B. COLI COMMUNIS IN WATER

By E. E. IRONS, Chicago.

Reprinted from the Proceedings of the Twenty-Eighth Annual Meeting, held at Indianapolis, Indiana, October 22, 23, 24, 25 and 26, 1900.

COLUMBUS, OHIO:
THE PERLIN PRINTING COMPANY.
1901.

SOME OBSERVATIONS ON METHODS FOR THE DETECTION OF B. COLI COMMUNIS IN WATER.

By E. E. IRONS, Chicago.

In the course of an extended examination of polluted river waters for the Sanitary District of Chicago, a number of methods for the detection of B. coli communis have been regularly employed, and still others experimented with. Four methods have been especially studied: (1) the dextrose fermentation tube, (2) the lactose fermentation tube, (3) the carbol-broth method, (4) the lactose plate.

For the dextrose and lactose fermentation tubes, broth was prepared from fresh meat from which the muscle-sugar had been extracted by Smith's method. One per cent. of sugar was then added to the broth. The initial reaction was made neutral to phenolphthalein, experiments having shown no appreciable difference between the results with this reaction and others slightly more acid. Experiments likewise showed no material difference between tubes containing 1 per cent. and 2 per cent. of sugar. Other factors of variation, over which we have no control, probably outweigh these smaller differences. The cultures were incubated at 38° C. for 48 hours, gas readings being taken after 24 hours and 48 hours. No material changes were observed if the incubation period was prolonged to three days. Tubes showing gas formation were removed from the thermostat, cooled to the temperature of the room and the absorption of CO_2 determined by the addition of a 2 per cent. solution of NaOH. Sometimes, particularly in the larger tubes, the absorption was incomplete after the first addition of NaOH, and more had to be added.

Carbol-broth was made by adding 1 c.c. of a 1 per cent. solution of carbolic acid in sterile water to tubes containing 9 c.c. of sterile common broth[1]. From such tubes, incubated 24 hours at 38° C., lactose plates were inoculated and placed in the thermostat for 24 hours. Fish cultures from resulting red colonies were made, and the growth reactions determined in milk, gelatin, potato, sugar-free broth for indol, and the dextrose fermentation tube.

Litmus-lactose-agar was prepared by adding to sugar-free agar, of a reaction 5 alkaline to phenolphthalein, 1 per cent. lactose and enough blue litmus solution to give a clear blue color. Plating with the water direct was carried on in the usual manner. The plates were incubated at 38° C. for 24 hours.

[1] Except where otherwise stated, media were prepared according to the "Procedures Recommended for the Study of Bacteria." Journ. Amer. Pub. Health Association., Jan., 1898

THE FERMENTATION TUBE.

Approximately 1100 determinations were made by the fermentation tube with dextrose and lactose. Lactose was used in hope that the trouble caused by B. cloacæ might be avoided, since B. cloacæ ferments lactose very slowly as compared with B. coli, but as will appear presently this expectation was not justified. The results obtained with dextrose broth seem to warrant the assumption that when the gas formula shows a marked excess of H over CO_2 (i. e., approximately a 2-1 formula) B. coli is generally present. A number of tubes giving such a formula were examined, and only rarely did we fail to isolate an organism closely approximating typical B. coli. Moreover when failure to isolate B. coli did occur, B. coli may still have been present, but overlooked among the large number of other colonies on the plate. Experiments with artificially mixed cultures of B. coli and B. cloacæ, freshly isolated from water, showed sometimes a coli, sometimes a cloacæ gas formula, but in the majority of cases B. coli gained the upper hand and an excess of H appeared in the tubes. When the fermentation tube is used for highly polluted waters, it occasionally happens that there is little or no formation of gas even though B. coli is probably present as shown by the typical gas formula in higher dilutions of the same water. This difficulty, however, may be obviated in large measure by using several dilutions where the pollution of the water is not approximately known.

The following table (Table A) gives the totals obtained in a series of 59 determinations in duplicate with dextrose and lactose broth. Each determination with dextrose was made simultaneously with the same dilution of the water as the corresponding lactose determination, and both were kept as nearly as possible under identical conditions. Both polluted and comparatively pure river waters were employed in this series.

TABLE A.

Dextrose Fermentation Tube.			Lactose Fermentation Tube.		
+	—	?	+	—	?
27	19	13	21	29	9

+ = total gas more than 20%; formula showing marked excess of H.
— = no gas; gas less than 10%.
? = total gas 10%-20%; total gas more than 20% with formula showing excess of CO_2.

The table shows that for general work dextrose is to be preferred to lactose. This is true for both polluted and unpolluted waters as is shown if the results obtained from the two classes of waters are tabulated separately. It is of course recognized that the B. enteritidis group ferments dextrose, but not lactose. However, germs of this class are probably rare in river waters.

THE CARBOL-BROTH METHOD.

Several hundred determinations were made by the use of carbol-broth followed by the lactose plate. The following table (Table B) shows the totals obtained in a series of duplicate tests made to ascertain the comparative value of the dextrose fermentation tube and the carbol-broth method on both polluted and relatively unpolluted river waters.

TABLE B.

	Dextrose Fermentation Tube.			Carbol-broth Method.		
	+	—	?	+	—	?
Polluted Waters	33	31	5	38	30	1
Relatively Unpolluted Waters	56	38	25	37	61	21

Dextrose fermentation tube: +, —, ?, as in Table A.

Carbol-broth method:

+ = red colonies on the lactose plate, which give the typical reactions of B. coli in (1) milk, (2) gelatine, (3) potato, (4) sugar-free broth for indol, (5) the dextrose fermentation tube.

— = no red colonies; red colonies do not give an excess of H in the dextrose fermentation tube, or they fail in the other reactions.

? = red colonies fail to give one or two of the first four typical reactions above.

From the results in Table B, it is apparent that the carbol-broth method gives a larger number of positive determinations for B. coli than does the dextrose fermentation tube, when polluted waters are tested. On the other hand, with relatively pure waters, the dextrose tube is shown to be a much more delicate test than the carbol-broth method.

THE LACTOSE PLATE.

Comparative experiments on the use of the lactose plate made in series with the dextrose fermentation tube as a control, resulted rather unfavorably for this method. In many cases the plates made with lactose agar having a reaction of 5 alkaline to phenolphthalein,

failed to show any red colonies, when the dextrose tube gave a 2-1 gas formula. When the alkalinity was reduced to 5 acid to phenolphthalein, many red colonies appeared which gave either an inverted gas formula, or, more often, no gas at all with dextrose, although the control dextrose tube showed the probable presence of B. coli.

In polluted waters where B. coli is present together with other slightly acid forming species, the excess of the acid of B. coli may so far neutralize the alkalinity of the medium that the weaker acid producers appear as red colonies. Hence there is a possibility of error in assuming that the number of red colonies on a plate indicates the presence of a proportionate amount of B. coli.

In general, results show that where the waters to be examined are much polluted, or where the amount of B. coli is small and the colony count large, the lactose plate for plating water direct is inferior to the dextrose fermentation tube.

Other methods were tried but they proved to be either impracticable, or no improvement on the foregoing. Elsner's medium was experimented with, but it failed to give as good results as lactose agar, besides being more difficult of preparation. The use of carbol-iodide-broth suggested itself as a possible means of differentiating the troublesome alkali producers which survive the preliminary incubation in carbol-broth. The iodide (1 per cent. KI) proved to add nothing appreciable to the inhibitive power of the carbolic acid. Experiments with various strengths of carbolic acid showed a dilution of 1-1000 to be the most advantageous.

The results of the work of which the foregoing experiments formed a part, suggest the following points:

1. When the dextrose tube method yields approximately 33 per cent. of CO_2, B. coli is almost invariably present.

2. For the direct inoculation of water, the dextrose fermentation tube is preferable to the lactose tube.

3. For polluted waters, incubation in carbol-broth followed by the lactose plate gives better results than the dextrose tube, while for relatively pure waters, the dextrose tube appears to be the more delicate.

4. When highly polluted waters are under examination, the lactose plate for the direct inoculation of water is less successful than either carbol-broth or the dextrose tube. In our experience this method has given the best results when applied to waters containing a small number of other bacteria in proportion to the amount of B. coli present.

I am indebted to Professor E. O. Jordan, under whose direction this work was done, for many helpful suggestions and criticisms.